The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 29 October 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
A
-7 OCT 2002
RECEIVED BY FAX

The Patent Office

07OCT02 E753750-1 D02973
P01/7700 0.00-0223187.6

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference — P101116GB

2. Patent application number *(The Patent Office will fill in this part)* — 0223187.6 — 7 OCT 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — Ludwig Institute for Cancer Research
Postfach 8024
ZURICH
Switzerland

Patents ADP number *(if you know it)* — 577155002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — Screen

5. Name of your agent *(if you have one)* — Harrison Goddard Foote

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — 31 St Saviourgate
YORK
YO1 8NQ

Patents ADP number *(if you know it)* — ~~[illegible]1001~~ 791423700Z

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

yes

0049181 07-Oct-02 03:42

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	14
Claim(s)	7
Abstract	1
Drawing(s)	10

8 ✓

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Harrison Goddard Foote Date 7 October 2002

12. Name and daytime telephone number of person to contact in the United Kingdom Robert C Docherty 01904 732120

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

SCREEN

The invention relates to a screening method to determine the susceptibility of a mammal, preferably a human, to abnormal development of the nervous system and including therapeutic methods and compositions for the treatment of neurodegenerative conditions which result in abnormal expression of a family of polypeptides which induce the apoptotic function of p53.

Apoptosis, or programmed cell death, is a process by which multi-cellular organisms regulate cell number and differentiation. The process is regulated by factors which either induce or prevent apoptosis. Inducers of apoptosis include Bcl-2 family members, caspase family members and their associated factors Apaf-1 and Fadd. Caspases are synthesised as proenzymes which become activated after proteolytic cleavage. The active caspase then induces many of the morphological and biochemical changes associated with apoptosis. Mitochondria play a pivotal role in the activation process through the release of pro-apoptotic factors such as cytochrome c, AIF and Diablo. The release from mitochondria is controlled by the Bcl-2 family of proteins; (e.g. Bcl-2 and Bcl-xl inhibit release; Bax and Bak induce release). WO9953051 discloses a cytokine dependent protein p21 which has pro-apoptotic activity. p21 is expressed in a cytokine dependent manner in myeloid/erthyroid cells. These cells are dependent on IL-3 for growth and in the absence of IL-3 the translation of p21 is induced resulting in apoptosis and cell death. p21 is a cytoplasmic protein which translocates to the outer mitochondrial membrane to induce pro-apoptotic activities.

Tumour suppressor proteins also have pro-apoptotic activities.

Tumour suppressor genes encode proteins which function to inhibit cell growth or division and are therefore important with respect to maintaining proliferation, growth and differentiation of normal cells. Mutations in tumour suppressor genes result in abnormal cell-cycle progression whereby the normal cell-cycle check points which

arrest the cell-cycle, when, for example, DNA is damaged, are ignored and damaged cells divide uncontrollably. The products of tumour suppressor genes function in all parts of the cell (e.g. cell surface, cytoplasm, nucleus) to prevent the passage of damaged cells through the cell- cycle (i.e. G1, S, G2, M and cytokinesis).

Arguably the tumour suppressor gene which has been the subject of the most intense research is p53. p53 encodes a protein which functions as a transcription factor and is a key regulator of the cell division cycle. It was discovered in 1978 as a protein shown to bind with affinity to the SV40 large T antigen. The p53 gene encodes a 393 amino acid polypeptide with a molecular weight of 53kDa. Genes regulated by the transcriptional activity of p53 contain a p53 recognition sequence in their 5' regions. These genes are activated when the cellular levels of p53 are elevated due to, for example DNA damage. Examples of genes which respond to p53 include, mdm2, Bax and PIG-3. Bax and PIG-3 are involved in one of the most important functions of p53, the induction of apoptosis.

In our co-pending application WO02/12325 we disclose, amongst other things, a new family of pro-apoptotic polypeptides which regulate the apoptotic activity of p53. We herein describe the involvement of this family in the development of the central nervous system (CNS) in mammals. Neonatal mice which are homozygous null for the ASPP 2 gene have abnormal development of the CNS. The cells of the CNS have abnormal patterns of division which results in abnormalities, particularly in the brain and retina, of developing neonatal mice. This is suggestive of the need for programmed cell death in the differentiation of the CNS and the involvement of the ASPP 2 family in regulating this process.

According to an aspect of the invention there is provided a method for the detection of a polypeptide in a cell or tissue sample which sample comprises a nerve cell or a nerve progenitor cell and wherein said polypeptide is a polypeptide which induces the apoptotic function of p53.

In a preferred method of the invention said polypeptide is selected from the group consisting of:

a) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

b) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (a); or

c) a polypeptide encoded by a nucleic acid molecule which is degenerate because of the genetic code to the nucleic acid molecule represented in (a) and (b); said method comprising the steps of;

i) providing a sample comprising a nerve cell or a nerve cell progenitor cell;

ii) contacting said sample with an agent which binds said polypeptide;

iii) detecting the presence of said polypeptide in said cell sample.

In a preferred method of the invention said polypeptide is encoded by a nucleic acid molecule which hybridises under stringent hybridisation conditions to the nucleic acid sequence as represented in Figure 1 or 2. Preferably said nucleic acid is represented by the nucleic acid sequence in Figure 1 or 2.

In a preferred embodiment of the invention said polypeptide is represented by the amino acid sequences in Figures 3 or 4 wherein said polypeptide is altered by addition, deletion or substitution of at least one amino acid residue.

In a preferred method of the invention said agent is an antibody which binds said polypeptide; preferably a polyclonal antibody.

In a further preferred method of the invention said antibody is a monoclonal antibody.

In a yet further preferred method of the invention said antibody is provided with means which enable the detection, either directly or indirectly, of the antibody bound to said polypeptide.

In a preferred method of the invention said detection means is selected from the group consisting of: an enzyme; a isotope label or a fluorescent label.

In an alternative preferred method of the invention said method is the detection of a nucleic acid molecule which encodes said polypeptide.

In an alternative preferred method of the invention said agent is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which encodes said polypeptide.

In a preferred method of the invention said nucleic acid molecule is at least one oligonucleotide molecule. Preferably a pair of oligonucleotide molecules adapted to bind said nucleic acid molecule which is to be detected. Preferably said method is a polymerase chain reaction method.

According to a further aspect of the application there is provided the use of a polypeptide selected from the group consisting of:

i) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;
ii) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (i); or
iii) a polypeptide encoded by a nucleic acid molecule which is degenerate because of the genetic code to the nucleic acid molecule represented in (i) and (ii).

for use in the manufacture of a medicament for use in the treatment of neurodegenerative diseases which result from a deficiency in said polypeptide.

In a preferred embodiment of the invention said polypeptide is represented by the amino acid sequence presented in Figure 3 or 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

We disclose that mice which do not express ASPP family members show abnormal neural development in the neonatal CNS and retina as a result of uncontrolled cell division. This strongly implicates ASPP induced apoptosis in the development of the CNS. In addition there are a number of neurodegenerative diseases in which apoptosis is implicated. For example, Alzheimer's disease, Parkinson's disease, and multiple sclerosis. It is possible that inappropriate expression of ASPP family members in these conditions could result in premature cell death of neurones. This theory is readily testable by methods established in the art.

In a preferred embodiment of the invention said polypeptide is encoded by a nucleic acid molecule. Preferably said nucleic acid molecule is part of a vector adapted for gene therapy.

The invention also contemplates gene therapy of neurodegenerative diseases. The procedure for performing *ex vivo* gene therapy is outlined in U.S. Patent 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction *in vitro* of a functional copy of a gene into a cell(s) of a subject which contains a defective copy of the gene, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO95/00654. *In vivo* gene therapy using vectors such as adenovirus, retroviruses, herpes virus, and targeted liposomes also is contemplated according to the invention.

According to a further aspect of the application there is provided the use of an antagonist which interacts with a polypeptide selected from the group consisting of:

i) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

ii) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (i); or

iii) a polypeptide encoded by a nucleic acid molecule which is degenerate because of the genetic code to the nucleic acid molecule represented in (i) and (ii).

for use in the manufacture of a medicament for use in the treatment of neurodegenerative diseases which result from abnormal expression of said polypeptide.

In a preferred embodiment of the invention said polypeptide is represented by the amino acid sequence presented in Figure 3 or 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

In a further preferred method of the invention said disease is selected from the group consisting of: Alzheimer's disease; Parkinson's disease; multiple sclerosis; retinopathies.

In a preferred embodiment of the invention said antagonist is an antibody or antibody part which binds said polypeptide. Preferably said antibody is a monoclonal antibody or binding part thereof.

Antibodies, also known as immunoglobulins, are protein molecules which usually have specificity for foreign molecules (antigens). Immunoglobulins (Ig) are a class of structurally related proteins consisting of two pairs of polypeptide chains, one pair of light (L) (low molecular weight) chain (κ or λ), and one pair of heavy (H) chains (γ, α, μ, δ and ε), all four linked together by disulphide bonds. Both H and L chains have regions that contribute to the binding of antigen and that are highly variable from one Ig molecule to another. In addition, H and L chains contain regions that are non-variable or constant.

The L chains consist of two domains. The carboxy-terminal domain is essentially identical among L chains of a given type and is referred to as the "constant" (C) region. The amino terminal domain varies from L chain to L chain and contributes to the binding site of the antibody. Because of its variability, it is referred to as the "variable" (V) region.

The H chains of Ig molecules are of several classes, α, μ, σ, α, and γ (of which there are several sub-classes). An assembled Ig molecule consisting of one or more units of two identical H and L chains, derives its name from the H chain that it possesses. Thus, there are five Ig isotypes: IgA, IgM, IgD, IgE and IgG (with four sub-classes based on the differences in the 'constant' regions of the H chains, i.e., IgG1, IgG2, IgG3 and IgG4). Further detail regarding antibody structure and their various functions can be found in, Using Antibodies: A laboratory manual, Cold Spring Harbour Laboratory Press.

In a preferred embodiment of the invention said fragment is a Fab fragment.

In a further preferred embodiment of the invention said antibody is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; and antibodies comprising CDR3 regions.

A modified antibody, or variant antibody, and reference antibody, may differ in amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination. Among preferred variants are those that vary from a reference polypeptide by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characteristics. The following non-limiting list of amino acids are considered conservative replacements (similar): a) alanine, serine, and threonine; b) glutamic acid and asparatic acid; c) asparagine and glutamine d) arginine and lysine; e) isoleucine, leucine, methionine and valine and f) phenylalanine, tyrosine and

tryptophan. Most highly preferred are variants which show enhanced biological activity.

Preferably said antibody is a humanised or chimeric antibody.

A chimeric antibody is produced by recombinant methods to contain the variable region of an antibody with an invariant or constant region of a human antibody.

A humanised antibody is produced by recombinant methods to combine the complementarity determining regions (CDRs) of an antibody with both the constant (C) regions and the framework regions from the variable (V) regions of a human antibody.

Chimeric antibodies are recombinant antibodies in which all of the V-regions of a mouse or rat antibody are combined with human antibody C-regions. Humanised antibodies are recombinant hybrid antibodies which fuse the complimentarity determining regions from a rodent antibody V-region with the framework regions from the human antibody V-regions. The C-regions from the human antibody are also used. The complimentarity determining regions (CDRs) are the regions within the N-terminal domain of both the heavy and light chain of the antibody to where the majority of the variation of the V-region is restricted. These regions form loops at the surface of the antibody molecule. These loops provide the binding surface between the antibody and antigen.

Antibodies from non-human animals provoke an immune response to the foreign antibody and its removal from the circulation. Both chimeric and humanised antibodies have reduced antigenicity when injected to a human subject because there is a reduced amount of rodent (i.e. foreign) antibody within the recombinant hybrid antibody, while the human antibody regions do not elicit an immune response. This results in a weaker immune response and a decrease in the clearance of the antibody. This is clearly desirable when using therapeutic antibodies in the treatment of human

diseases. Humanised antibodies are designed to have less "foreign" antibody regions and are therefore thought to be less immunogenic than chimeric antibodies.

In an alternative preferred embodiment of the invention said antagonist is a nucleic acid molecule.

In a preferred embodiment of the invention said nucleic acid molecule is selected from the group consisting of an antisense molecule or an inhibitory RNA molecule designed with reference to Figure 1 or 2.

As used herein, the term "antisense molecule" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridises under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridisation with the target gene or transcript. Those skilled in the art will recognise that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridise substantially more to the target sequence than to any other sequence in the target cell under physiological conditions. Based upon the ASPP-2 nucleic acid sequences provided herein, or upon allelic or homologous genomic and/or cDNA sequences, one of skill in the art can easily choose and synthesise any of a number of appropriate antisense molecules for use in accordance with the present invention. For example, a "gene walk" comprising a series of oligonucleotides of 15-30 nucleotides spanning the length of ASPP-2 nucleic acid can be prepared, followed by testing for inhibition of the corresponding

ASPP-2 expression. Optionally, gaps of 5-10 nucleotides can be left between the oligonucleotides to reduce the number of oligonucleotides synthesised and tested.

In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides (Wagner et al., *Nature Biotechnol.* 14:840-844, 1996). Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which proteins are not expected to bind. Finally, although ASPP1/ASPP-2 cDNA sequences are disclosed herein, one of ordinary skill in the art may easily derive the genomic DNA corresponding to these cDNAs. Thus, the present invention also provides for antisense oligonucleotides which are complementary to ASPP1/ASPP-2 genomic DNA. Similarly, antisense to allelic or homologous cDNAs and genomic DNAs are enabled without undue experimentation.

In one set of embodiments, the antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognised methods which may be carried out manually or by an automated synthesiser. They also may be produced recombinantly by vectors.

A recent technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA (RNAi), into a cell which results in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the gene which is to be ablated.

Recent studies suggest that RNAi molecules ranging from 100-1000bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

In a preferred embodiment of the invention there is provided a transcription cassette comprising an nucleic acid sequence operatively linked to a promoter which promoter transcribes said nucleic acid molecule to produce an antisense nucleic acid molecule, said sequence selected from the group consisting of:

i) a nucleic acid sequence, or part thereof, as represented in Figure 1 or 2;

ii) a nucleic acid sequence which hybridises to the sense sequence presented in Figure 1 or 2 and which encodes a polypeptide with anti-apoptotic activity.

In a preferred embodiment of the invention said cassette is part of a vector.

In a further preferred embodiment of the invention there is provided a transcription cassette comprising a nucleic acid molecule, or part thereof, selected from the group consisting of:

i) a nucleic acid molecule represented by the nucleic acid sequence in Figure 1 or 2;

ii) a nucleic acid molecule which hybridises to the sequence in (i) above and which encodes a polypeptide with anti-apoptotic activity; or

iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

In a preferred embodiment of the invention said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

In a further preferred embodiment of the invention said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

In a preferred embodiment of the invention said first and second parts are linked by at least one nucleotide base.

In a preferred embodiment of the invention said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 nucleotide bases.

In a further preferred embodiment of the invention the length of the RNAi molecule is between 100bp-1000bp. More preferably still the length of RNAi is selected from 100bp; 200bp; 300bp; 400bp; 500bp; 600bp; 700bp; 800bp; 900bp; or 1000bp. More preferably still said RNAi is at least 1000bp.

In an alternative preferred embodiment of the invention the RNAi molecule is between 15bp and 25bp, preferably said molecule is 21bp.

In a preferred embodiment of the invention said cassette is part of a vector.

According to a further aspect of the invention there is provided method to screen for agents which modulate the activity of a polypeptide which induces the apoptotic function of p53 comprising the steps of:

i) providing a cell sample comprising a nerve cell or nerve progenitor cell;
ii) contacting said sample with an agent to be tested; and
iii) monitoring effect of said agent on the presence and/or activity of said polypeptide.

In a preferred method of the invention said polypeptide is selected from the group consisting of:

a) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;
b) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (a); or
c) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (a) and (b).

In a further preferred method of the invention said agent is an antagonist of said polypeptide.

In an alternative preferred method of the invention said agent is an agonist of said polypeptide.

An embodiment of the invention will now be described by example only and with reference to the following figures:

Figure 1 is a DNA sequence which encodes ASPP1;

Figure 2 is a DNA sequence which encodes ASPP2;

Figure 3 is the amino acid sequence of ASPP1;

Figure 4 is the amino acid sequence of ASPP2;

Figure 5 illustrates that the percentage of ASPP2 null mice born is normal;

Figure 6 illustrates the malformation of ASPP2 null embryos;

Figure 7 illustrates that ASPP2 null mice die before weaning;

Figure 8 illustrates eye malformation in 13.5 day embryos in ASPP2 (+/-) and ASPP2 (+/+) mice;

Figure 9 illustrates brain malformation 13.5 day embryos in ASPP2 (+/-) and ASPP2 (+/+) mice; and

Figure 10 illustrates abnormal cell growth in the brain of an ASPP2 null embryo.

CLAIMS

1 A method for the detection of a polypeptide in a cell or tissue sample which sample comprises a nerve cell or a nerve progenitor cell and wherein said polypeptide is a polypeptide which induces the apoptotic function of p53.

2. A method according to Claim 1 wherein said polypeptide is selected from the group consisting of:

a) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

b) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (a); or

c) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (a) and (b); said method comprising the steps of;

i) providing a sample comprising a nerve cell or a nerve cell progenitor cell;

ii) contacting said sample with an agent which binds said polypeptide;

iii) detecting the presence of said polypeptide in said cell sample.

3. A method according to Claim 2 wherein said polypeptide is encoded by a nucleic acid molecule which hybridises under stringent hybridisation conditions to the nucleic acid sequence as represented in Figure 1 or 2.

4. A method according to Claim 3 wherein said nucleic acid is represented by the nucleic acid sequence in Figure 1 or 2.

5. A method according to any of Claims 2-4 wherein said polypeptide is represented by the amino acid sequence in Figures 3 and 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

6. A method according to any of Claims 1-5 wherein said agent is an antibody which binds said polypeptide.

7. A method according to Claim 6 wherein said antibody is a polyclonal antibody.

8. A method according to Claim 6 wherein said antibody is a monoclonal antibody.

9. A method according to any of Claims 6-8 wherein said antibody is provided with means which enable the detection of the antibody bound to said polypeptide.

10. A method according to Claim 9 wherein said detection means is selected from the group consisting of: an enzyme; a isotope label or a fluorescent label.

11. A method according to any of Claims 1-5 wherein said method is the detection of a nucleic acid molecule which encodes said polypeptide.

12. A method according to Claim 11 wherein said agent is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which encodes said polypeptide.

13. A method according to Claim 12 wherein said nucleic acid molecule is at least one oligonucleotide molecule.

14. A method according to Claim 13 wherein said nucleic acid molecule is a pair of oligonucleotide molecules adapted to bind said nucleic acid molecule which is to be detected.

15. A method according to Claim 14 wherein said method is a polymersase chain reaction method.

16. The use of a polypeptide selected from the group consisting of:

i) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

ii) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (i); or

iii) a polypeptide encoded by a nucleic acid molecule which is degenerate because of the genetic code to the nucleic acid molecule represented in (i) and (ii).

for use in the manufacture of a medicament for use in the treatment of neurodegenerative diseases which result from a deficiency in said polypeptide.

17. A method according to Claim 16 wherein said polypeptide is encoded encoded by a nucleic acid molecule.

18. A method according to Claim 16 or 17 wherein said polypeptide is represented by the amino acid sequence in Figures 3 and 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

19. A method according to Claim 17 wherein said nucleic acid molecule is part of a vector adapted for gene therapy.

20. The use of an antagonist which interacts with a polypeptide selected from the group consisting of:

i) a polypeptide encoded by a nucleic acid molecule as represented by the sequence in Figure 1 or 2;

ii) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (i); or

iii) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (i) and (ii).

for use in the manufacture of a medicament for use in the treatment of neurodegenerative diseases which result from abnormal expression of said polypeptide.

21. Use according to Claim 20 wherein said polypeptide is represented by the amino acid sequence in Figures 3 and 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

22. Use according to Claim 20 or 21 wherein said disease is selected from the group consisting of: Alzheimer's disease; Parkinson's disease; multiple sclerosis; or a retinopathy.

23. Use according to any of Claims 20-22 wherein said antagonist is an antibody or antibody part which binds said polypeptide.

24. Use according to Claim 23 wherein said antibody is a monoclonal antibody or binding part thereof.

25. Use according to Claim 23 or 24 wherein said fragment is a Fab fragment.

26. Use according to Claim 25 wherein said fragment is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; and CDR3 regions.

27. Use according to any of Claims 24-26 wherein said antibody is a humanised.

28. Use according to any of Claims 24-26 wherein said antibody is a chimeric antibody.

29. Use according to Claim 20 wherein said antagonist is a nucleic acid molecule.

30. Use according to Claim 29 wherein said nucleic acid molecule is a transcription cassette comprising an nucleic acid molecule operatively linked to a promoter which promoter transcribes said nucleic acid molecule to produce an antisense nucleic acid molecule, said sequence selected from the group consisting of:

i) a nucleic acid sequence, or part thereof, as represented in Figure 1 or 2;

ii) a nucleic acid sequence which hybridises to the sense sequence presented in Figure 1 or 2 and which encodes a polypeptide with anti-apoptotic activity.

31 Use according to Claim 30 wherein said cassette is part of a vector.

32. Use according to Claim 29 wherein said nucleic acid molecule comprises a transcription cassette wherein said a nucleic acid molecule, or part thereof, selected from the group consisting of:

i) a nucleic acid molecule represented by the nucleic acid sequence in Figure 1 or 2;

ii) a nucleic acid molecule which hybridises to the sequences in (i) above and which encodes a polypeptide with anti-apoptotic activity; or

iii) a nucleic acid molecule which is degenerate as a consequence of the genetic code to the sequences defined in (i) and/or (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

33. Use according to Claim 32 wherein said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

34. Use according to Claim 32 wherein said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

35. Use according to Claim 34 wherein said first and second parts are linked by at least one nucleotide base.

36. Use according to Claim 35 wherein said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 nucleotide bases.

37. Use according to any of Claims 32-36 wherein the length of said RNAi molecule is between 100bp-1000bp.

38. Use according to Claim 37 wherein the length of said RNAi molecule is selected from at least 100bp; 200bp; 300bp; 400bp; 500bp; 600bp; 700bp; 800bp; 900bp; or 1000bp.

39. Use according to any of Claims 32-36 wherein said RNAi is at least 1000bp in length.

40 Use according to any of Claims 32-36 wherein said RNAi molecule is between 15bp and 25bp in length.

41. Use according to Claim 40 wherein said RNAi molecule is 21bp in length.

42. Use according to any of Claims 32-41 wherein said cassette is part of a vector.

43. A method to screen for agents which modulate the activity of a polypeptide which induces the apoptotic function of p53 comprising the steps of:

iv) providing a cell sample comprising a nerve cell or nerve progenitor cell;

v) contacting said sample with an agent to be tested; and

vi) monitoring effect of said agent on the presence and/or activity of said polypeptide.

44. A method according to Claim 43 wherein said polypeptide is selected from the group consisting of:

d) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

e) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (a); or

f) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (a) and (b).

44. A method according to Claim 43 or 44 wherein said agent is an antagonist of said polypeptide.

45. A method according to Claim 43 or 44 wherein said agent is an agonist of said polypeptide.

Abstract

Screen

The invention relates to a screening method to determine the susceptibility of a mammal, preferably a human, to abnormal development of the nervous system and including therapeutic methods and compositions for the treatment of neurodegenerative conditions which result in abnormal expression of a family of polypeptides which induce the apoptotic function of p53.

Figure 1

GAGCCCCGCATCCCGCCGCAGCTGCCGCCTCGCCGCGGCCGGGCCGGAGAGCACGGCGGCGGGAGCGCGGCCTTAGGAGG
CGGCCGGAGCGGTGGGCACAGCTCGGCGCGGAGCGTCCTGTCAGGCGGCGGCCGAGGGCGTCGCGGACTCTCCCCGCGAT
GATGCCGATGATATTAACTGTTTTCTTGAGCAACAATGAACAGATTTTAACAGAAGTTCCTATAACACCGGAAACAACCT
GTCGAGATGTTGTAGAATTTTGCAAGGAACCTGGAGAAGGCAGCTGCCATTTAGCTGAAGTGTGGAGGGGAAATGAACGT
CCCATACCCTTTGATCATATGATGTACGAACATCTTCAGATATGGGGTCCACGGAGGGAAGAAGTGAAATTTTTCCTTCG
ACACGAGGACTCCCCAACTGAGAACAGTGAACAAGGTGGCCGTCAGACCCAAGAGCAACGAACTCAGAGAAATGTAATAA
ATGTACCTGGAGATAAACGTACTGAATATGGGTTGGAATCCACGTGTTGAACTTACCCTCTCAGAGCTCCAAGATATG
GCAGCTAGGCAACAGCAGCAGATTGAAAATCAGCAGCAGATGTTGGTTGCCAAGGAACAGCGTTTACATTTTCTAAAGCA
ACAGGAGCGCCGTCAGCAGCAGTCTATTTCTGAAAATGAAAAGCTTCAGAAATTGAAAGAACGAGTTGAAGCCCAGGAGA
ACAAGCTGAAGAAAATTCGTGCAATGAGAGGACAAGTCGACTACAGCAAAATCATGAACGGCAATCTGTCTGCTGAAATA
GAAAGGTTCAGTGCCATGTTCCAGGAAAAGAAGCAGGAAGTACAGACTGCAATTTTAAGGGTTGATCAGCTTAGTCAGCA
ATTGGAAGATTTAAAGAAAGGAAAACTGAATGGGTTCCAGTCTTACAATGGCAAATTGACGGGACCAGCGGCGGTGGAGT
TAAAAAGACTGTACCAAGAACTACAGATTCGTAACCAACTTAACCAGGAACAAAATTCAAAACTTCAGCAGCAGAAGGAA
CTCTTAAATAAGCGCAACATGGAGGTGGCCATGATGGACAAGCGAATCAGTGAACTGCGTGAACGTCTCTATGGAAAAA
AATTCAGCTGAACCGTGTGAATGGCACGTCATCACCACAGTCCCCTCTGAGCACATCGGGCAGGGTCGCTGCTGTGGGC
CTTATATCCAGGTTCCCAGTGCCGGAAGCTTTCCTGTGCTGGGGGACCCTATAAAGCCCCAGTCTCTCAGTATTGCCTCA
AATGCTGCTCATGGAAGATCCAAATCCGCTAATGATGGAAACTGGCCAACATTAAAACAGAATTCTAGCTCTTCCGTGAA
ACCAGTGCAGGTGGCCGGTGCAGACTGGAAGGATCCGAGCGTGGAGGGGTCTGTCAAGCAGGGCACTGTCTCCAGCCAGC
CTGTGCCCTTCTCAGCACTGGGACCCACGGAGAAGCCGGGCATCGAGATTGGTAAAGTGCCACCTCCCATCCCGGGTGTA
GGCAAGCAGCTGCCTCCAAGCTATGGGACATACCCAAGTCCTACACCTCTGGGTCCTGGGTCGACAAGCTCCCTGGAAAG
GAGGAAGGAAGGCAGCTTGCCCAGGCCCAGTGCAGGCCTGCCAAGTCGACAGAGGCCCACCCTGCTGCCCGCCACAGGCA
GCACCCCCCAGCCAGGCTCCTCACAACAGATTCAGCAGAGGATTTCCGTACCGCCAAGTCCCACGTACCCGCCAGCGGGA
CCACCTGCATTTCCAGCTGGGGACAGCAAGCCTGAACTCCCACTGACAGTGGCCATTAGGCCTTTCCTGGCTGATAAAGG
GTCAAGGCCACAGTCTCCCAGGAAAGGACCCCAGACAGTGAATTCAAGTTCCATATACTCCATGTACCTCCAGCAAGCCA
CACCACCTAAGAATTACCAGCCGGCAGCACACAGCGCCTTAAATAAGTCAGTTAAAGCAGTGTATGGTAAGCCCGTTTTA
CCTTCGGGTTCAACCTCTCCATCGCCGCTGCCGTTTCTTCACGGGTCACTGTCCACGGGCACACCACAGCCTCAGCCACC
TTCAGAAAGTACTGAGAAAGAGCCTGAGCAGGATGGCCCCGCCGCCCCCGCAGATGGCAGCACCGTGGAGAGCCTGCCAC
GGCCACTCAGCCCCACCAAGCTCACGCCCATCGTGCATTCGCCACTGCGCTACCAGAGTGATGCAGACCTGGAGGCCCTC
CGCAGGAAGCTGGCCAACGCGCCCCGGCCCCTGAAAAAGCGCAGCTCCATCACAGAGCCCGAGGGCCCCGGCGGGCCCAA
CATCCAGAAGCTGCTGTACCAGCGCTTCAACACCCTGGCCGGTGGCATGGAGGGCACCCCTTTCTACCAGCCCAGCCCCT
CCCAGGACTTCATGGGCACCTTGGCCGATGTGGACAATGGAAACACCAATGCCAATGGAAACCTGGAAGAGCTCCCCCCT
GCCCAGCCCACAGCCCCACTCCCCGCTGAGCCTGCCCCGTCATCAGATGCCAATGATAATGAGTTACCTTCCCCCGAACC
AGAGGAGCTCATCTGTCCCCAAACCACCCACCAAACTGCCGAGCCGGCAGAGGACAATAACAACAACGTGGCCACGGTCC
CCACCACGGAGCAGATCCCGAGTCCTGTGGCTGAGGCCCCATCTCCAGGGGAAGAGCAGGTCCCTCCAGCACCTGTTCCC
CCTGCCAGCCACCCTCCTGCCACCTCCACGAACAAGCGGACCAACTTGAAGAAGCCCAACTCGGAGCGGACGGGGCACGG
GCTGAGAGTCCGGTTTAACCCCCTGGCACTGCTCCTAGACGCGTCTCTGGAAGGAGAGTTCGATCTGGTGCAGAGGATCA
TCTATGAGGTGGAAGATCCCAGCAAGCCCAACGATGAAGGGATCACCCCACTGCACAACGCCGTCTGCGCCGGCCACCAT
CACATCGTGAAGTTCCTGCTGGATTTTGGTGTCAACGTGAATGCTGCTGATAGTGATGGATGGACGCCGCTGCACTGCGC
TGCCTCTTGTAACAGCGTTCACCTCTGCAAACAGCTGGTGGAGAGTGGTGCCGCCATTTTTGCCTCAACCATAAGCGACA
TTGAAACTGCTGCAGACAAGTGTGAGGAGATGGAGGAAGGCTACATCCAGTGCTCCCAGTTTCTATATGGGGTGCAGGAA
AAGCTGGGTGTGATGAACAAAGGTGTGGCGTATGCTCTGTGGGACTACGAGGCCCAGAACAGTGACGAGCTGTCCTTCCA
CGAAGGGGACGCCCTCACCATCCTGAGGCGCAAGGACGAAAGCGAGACTGAGTGGTGGTGGGCTCGCCTTGGAGACCGGG
AGGGCTATGTGCCCAAAAACCTGCTGGGGCTGTATCCACGGATCAAACCCCGACAGCGAACACTCGCCTGAACTTCCTTT
TGGAGCACCGCATGGTCTTGCCAGCTACCAGGAGCCACTTAAGAGATTATTGTGCTGTTTTCCAGGAAAGCTGCAGCTAG
AAAATGGTCTTAATGGTGCTCACTTTAGCAGACAGCGTCCACAATGTGAATCCTACAGTTTCCAGGTGAGGCCCTTTCTC
CAGTTTGCCCATTAACTGGGAGAGGTACTTTGGCCTCCAAGGACTGAATTTTGCCAATTACTATAAATCCAAATAAATAC
CCACTTTCAAAACACCCACCCCTCTTGCCATTAAGAAGTCCCATAACCCCCGGTTGGTTGCCAGTGAAGACAGAAGCTCT
TACTGACTTGGCCCCGAGGCCATCACCCCCTCCAGCAGTGAACACTGTCCGCCGCTGTGAGGCCTGCTCCCCTGCGACCG
CCCTGCCCCCCGTCACCGAATCGGACACTCATCCTTTCTCACACTTCCCACACATGATCCTTCTTCCCTTCATCACCAAA
GGAGCCTCTGTATGGAAACATGTCCAGTGTTGCTGCCCAGTGTGTATGCCTCCCAGTACCCACTCTGCTCGGCCGCCTTG
GGGGTTCCGCTTCCTGTTCCAGTTCACCTAAAGGCTGATTGTGCAGGCCCAGCACTGTGGCTGGACTGCCGCGCCACGGG
CACCAGGACCCCTAAGACCAAGTGACAACTGGGAGAGCCTCAGCATATACTCTTCTCCTCCGATCTCACAGCCTGTCATG
CTGCTCAGTGTGGTTCTCACCCCTGCAAGCTCAAATTCAGTTCCCTGAATGGAGTCAGGTGCTGGAGGCCGTGGCAGCGG
AGGGTGGTTGGGGTTGGGGCTGGGGTGGACTGGTGTGAGGGCAGACCAGGGCCAGGTAGACGGGGCTGTTTGGTGCCTG
AAGGATGGCAGACGCCTGGTGTCAGGAGGGGCCGCCACCAAGGAGCAGCAGCTGGGGCAGAGGAGCTGGGGTCAGGGGCC
ACCCCTCTCTGCCGATCTCCCTGCCTGGGCTGGCTGTGAGGCCACCTTTGTCCCAGGCCCAGCCTCAAGGCAAGGAGGGC
GCTTCACTGAGGTGTGAATTGTACGTACAGGCTTTTTATATACCAAAAGTATTTTTTGACTAGACCATTCAAAGCTACCC
GAACTATGTTGGAAATTTTTTTTTTCTCATTAAAATACAGGCCCTTAGGCTCTATTTTTCATGTATGAGTCGTGTGTAA
TTTATGTAAAAATGTGTGTACAGACTCACTGATGCAGCACTGTAGCCCATCACCTTGGAGCACTGACTGTACATAGTGTG
GTGAAGAAAAGTGAACGCCCTTGTAGAGCAGCCCGACCACAGGAGCATGGCCGCTGCCAGCCCAGACGCTGCTGACGCTG
TGTAAATGTGCACAATAAACCCGTCTCACCCCGG

Figure 2

GTCACGAGCGTCGAAGAGACAAAGCCGCGTCAGGGGGCCCGGCCGGGGCGGGGAGCCCGGGGCTTGTTGGTGCCCCAGC
CCGCGCGGAGGGCCCTTCGGACCCGCGCGCCGCCGCTGCCGCCGCCGCCTCGCAACAGGTCCGGGCGGCCTCGCTCT
CCGCTCCCCTCCCCCGCATCCGCGACCCTCCGGGGCACCTCAGCTCGGCCGGGGCCGCAGTCTGGCCACCCGCTTCCATG
CGGTTCGGGTCCAAGATGATGCCGATGTTTCTTACCGTGTATCTCAGTAACAATGAGCAGCACTTCACAGAAGTTCCAGT
TACTCCAGAAACAATATGCAGAGACGTGGTGGATCTGTGCAAAGAACCCGGCGAGAGTGATTGCCATTTGGCTGAAGTGT
GGTGTGGCTCTGAACGTCCAGTTGCGGATAATGAGCGAATGTTTGATGTTCTTCAACGATTTGGAAGTCAGAGGAACGAA
GTTCGCTTCTTCCTTCGTCATGAACGCCCCCCTGGCAGGGACATTGTGAGTGGACCAAGATCTCAGGATCCAAGTTTAAA
AAGAAATGGTGTAAAAGTTCCTGGTGAATATCGAAGAAAGGAGAACGGTGTTAATAGTCCTAGGATGGATCTGACTCTTG
CTGAACTTCAGGAAATGGCATCTCGCCAGCAGCAACAGATTGAAGCCCAGCAACAATTGCTGGCAACTAAGGAACAGCGC
TTAAAGTTTTTGAAACAACAAGATCAGCGACAACAGCAACAAGTTGCTGAGCAGGAGAAACTTAAAAGGCTAAAAGAAAT
AGCTGAGAATCAGGAAGCTAAGCTAAAAAAAGTGAGAGCACTTAAAGGCCACGTGGAACAGAAGAGACTAAGCAATGGGA
AACTTGTGGAGGAAATTGAACAGATGAATAATTTGTTCCAGCAAAAACAGAGGGAGCTCGTCCTGGCTGTGTCAAAAGTA
GAAGAACTGACCAGGCAGCTAGAGATGCTCAAGAACGGCAGGATCGACAGCCACCATGACAATCAGTCTGCAGTGGCTGA
GCTTGATCGCCTCTATAAGGAGCTGCAGCTAAGAAACAAATTGAATCAAGAGCAGAATGCCAAGCTACAACAACAGAGGG
AGTGTTTGAATAAGCGTAATTCAGAAGTGGCAGTCATGGATAAGCGTGTTAATGAGCTGAGGGACCGGCTGTGGAAGAAG
AAGGCAGCTCTACAGCAAAAAGAAAATCTACCAGTTTCATCTGATGGAAATCTTCCCCAGCAAGCCGCGTCAGCCCCAAG
CCGTGTGGCTGCAGTAGGTCCCTATATCCAGTCGTCTACTATGCCTCGGATGCCCTCAAGGCCTGAATTGCTGGTGAAGC
CAGCCCTGCCGGATGGTTCCTTGGTCATTCAGGCTTCAGAGGGGCCGATGAAAATACAGACACTGCCCAACATGAGATCT
GGGGCTGCTTCACAAACTAAAGGCTCTAAAATCCATCCAGTTGGCCCTGATTGGAGTCCTTCAAATGCAGATCTTTTCCC
AAGCCAAGGCTCTGCTTCTGTACCTCAAAGCACTGGGAATGCTCTGGATCAAGTTGATGATGGAGAGGTTCCGCTGAGGG
AGAAAGAGAAGAAAGTGCGTCCGTTCTCAATGTTTGATGCAGTAGACCAGTCCAATGCCCCACCTTCCTTTGGTACTCTG
AGGAAGAACCAGAGCAGTGAAGATATCTTGCGGGATGCTCAGGTTGCAAATAAAAATGTGGCTAAAGTACCACCTCCTGT
TCCTACAAAACCAAAACAGATTAATTTGCCTTATTTTGGACAAACTAATCAGCCACCTTCAGACATTAAGCCAGACGGAA
GTTCTCAGCAGTTGTCAACAGTTGTTCCGTCCATGGAACTAAACCAAAACCAGCAGGGCAGCAGCCGAGAGTGCTGCTA
TCTCCCAGCATACCTTCGGTTGGCCAAGACCAGACCCTTTCTCCAGGTTCTAAGCAAGAAAGTCCACCTGCTGCTGCCGT
CCGGCCCTTTACTCCCCAGCCTTCCAAAGACACCTTACTTCCACCCTTCAGAAAACCCCAGACCGTGGCAGCAAGTTCAA
TATATTCCATGTATACGCAACAGCAGGCGCCAGGAAAAAACTTCCAGCAGGCTGTGCAGAGCGCGTTGACCAAGACTCAT
ACCAGAGGGCCACACTTTTCAAGTGTATATGGTAAGCCTGTAATTGCTGCTGCCCAGAATCAACAGCAGCACCCAGAGAA
CATTTATTCCAATAGCCAGGGCAAGCCTGGCAGTCCAGAACCTGAAACAGAGCCTGTTTCTTCAGTTCAGGAGAACCATG
AAAACGAAAGAATTCCTCGGCCACTCAGCCCAACTAAATTACTGCCTTTCTTATCTAATCCTTACCGAAACCAGAGTGAT
GCTGACCTAGAAGCCTTACGAAAGAAACTGTCTAACGCACCAAGGCCTCTAAAGAAACGTAGTTCTATTACAGAGCCAGA
GGGTCCTAATGGGCCAAATATTCAGAAGCTTTTATATCAGAGGACCACCATAGCGGCCATGGAGACCATCTCTGTCCCAT
CATACCCATCCAAGTCAGCTTCTGTGACTGCCAGCTCAGAAAGCCCAGTAGAAATCCAGAATCCATATTTACATGTGGAG
CCCGAAAAGGAGGTGGTCTCTCTGGTTCCTGAATCATTGTCCCCAGAGGATGTGGGGAATGCCAGTACAGAGAACAGTGA
CATGCCAGCTCCTTCTCCAGGCCTTGATTATGAGCCTGAGGGAGTCCCAGACAACAGCCCAAATCTCCAGAATAACCCAG
AAGAACCAAATCCAGAGGCTCCACATGTGCTTGATGTGTACCTGGAGGAGTACCCTCCATACCCACCCCCACCATACCCA
TCTGGGGAGCCTGAAGGGCCCGGAGAAGACTCGGTGAGCATGCGCCCGCCTGAAATCACCGGGCAGGTCTCTCTGCCTCC
TGGTAAAAGGACAAACTTGCGTAAAACTGGCTCAGAGCGTATCGCTCATGGAATGAGGGTGAAATTCAACCCCCTTGCTT
TACTGCTAGATTCGTCTTTGGAGGGAGAATTTGACCTTGTACAGAGAATTATTTATGAGGTTGATGACCCAAGCCTCCCC
AATGATGAAGGCATCACGGCTCTTCACAATGCTGTGTGTGCAGGCCACACAGAAATCGTTAAGTTCCTGGTACAGTTTGG
TGTAAATGTAAATGCTGCTGATAGTGATGGATGGACTCCATTACATTGTGCTGCCTCATGTAACAACGTCCAAGTGTGTA
AGTTTTTGGTGGAGTCAGGAGCCGGCTGTGTTTGCCATGACCTACAGTGACATGCAGACTGCTGCAGATAAGTGCGAGGAA
ATGGAGGAAGGCTACACTCAGTGGTCCCAATTTCTTTATGGAGTTCAGGAGAAGATGGGCATAATGAATAAAGGAGTCAT
TTATGCGCTTTGGGATTATGAACCTCAGAATGATGATGAGCTGCCCATGAAAGAAGGAGACTGCATGACAATCATCCACA
GGGAAGACGAAGATGAAATCGAATGGTGGTGGGCGCGCCTTAATGATAAGGAGGGATATGTTCCACGTAACTTGCTGGGA
CTGTACCCAAGAATTAAACCAAGACAAAGGAGCTTGGCCTGAAACTTCCACACAGAATTTTAGTCAATGAAGAATTAATC
TCTGTTAAGAAGAAGTAATACGATTATTTTTGGCAAAAATTTCACAAGACTTATTTTAATGACAATGTAGCTTGAAAGCG
ATGAAGAATGTCTCTAGAAAGAGAATGAAGGATTGAAGAATTCACCATTAGAGGACATTTAGCGTGATGAAATAAAGCATC
TACGTCAGCAGGCCATACTGTGTTGGGGCAAAGGTGTCCCGTGTAGCACTCAGATAAGTATACAGCGACAATCCTGTTTT
CTACAAGAATCCTGTCTAGTAAATAGGATCATTTATTGGGCAGTTGGGAAATCAGCTCTCTGTCCTGTTGAGTGTTTTCA
GCAGCTGCTCCTAAACCAGTCCTCCTGCCAGAAAGGACCAGTGCCGTCACATCGCTGTCTCTGATTGTCCCCGGCACCAG
CAGGCCTTGGGGCTCACTGAAGGCTCGAAGGCACTGCACACCTTGTATATTGTCAGTGAAGAACGTTAGTTGGTTGTCAG
TGAACAATAACTTTATTATATGAGTTTTTGTAGCATCTTAAGAATTATACATATGTTTGAAATATTGAAACTAAGCTACA
GTACCAGTAATTAGATGTAGAATCTTGTTTGTAGGCTGAATTTTAATCTGTATTTATTGTCTTTTGTATCTCAGAAATTA
GAAACTTGCTACAGAGTTACCCGTAATATTTGTCAAGATCATAGCTGACTTTAAAAACAGTTGTAATAAACTTTTTGATG
CT

Figure 3

MMPMILTVFLSNNEQILTEVPITPETTCRDVVEFCKEPGEGSCHLAEVWRGNERPIPFDHMMYEHLQIWGPRREEVKFFL
RHEDSPTENSEQGGRQTQEQRTQRNVINVPGDKRTEYGVGNPRVELTLSELQDMAARQQQQIENQQQMLVAKEQRLHFLK
QQERRQQQSISENEKLQKLKERVEAQENKLKKIRAMRGQVDYSKIMNGNLSAEIERPSAMFQEKKQEVQTAILRVDQLSQ
QLEDLKKGKLNGPQSYNGKLTGPAAVELKRLYQELQIRNQLNQEQNSKLQQQKELLNKRNMEVAMMDKRISELRERLYGK
KIQLNRVNGTSSPQSPLSTSGRVAAVGPYIQVPSAGSFPVLGDPIKPQSLSIASNAAHGRSKSANDGNWPTLKQNSSSSV
KPVQVAGADWKDPSVEGSVKQGTVSSQPVPFSALGPTEKPGIEIGKVPPPIPGVGKQLPPSYGTYPSPTPLGPGSTSSLE
ERKEGSLPRPSAGLPSRQRPTLLPATGSTPQPGSSQQIQQRISVPPSPTYPPAGPPAFPAGDSKPELPLTVAIRPFLADK
GSRPQSPRKGPQTVNSSSIYSMYLQQATPPKNYQPAAHSALNKSVKAVYGKPVLPSGSTSPSPLPFLHGSLSTGTPQPQP
PSESTEKEPEQDGPAAPADGSTVESLPRPLSPTKLTPIVHSPLRYQSDADLEALRRKLANAPRPLKKRSSITEPEGPGGP
NIQKLLYQRFNTLAGGMEGTPFYQPSPSQDFMGTLADVDNGNTNANGNLEELPPAQPTAPLPAEPAPSSDANDNELPSPE
PEELICPQTTHQTAEPAEDNNNNVATVPTTEQIPSPVAEAPSPGEEQVPPAPLPPASHPPATSTNKRTNLKKPNSERTGH
GLRVRFNPLALLLDASLEGEFDLVQRIIYEVEDPSKPNDEGITPLHNAVCAGHHHIVKFLLDFGVNVNAADSDGWTPLHC
AASCNSVHLCKQLVESGAAIFASTISDIETAADKCEEMEEGYIQCSQFLYGVQEKLGVMNKGVAYALWDYEAQNSDELSF
HEGDALTILRRKDESETEWWWARLGDREGYVPKNLLGLYPRIKPRQRTLA

Figure 4

MMPMFLTVYLSNNEQHFTEVPVTPETICRDVVDLCKEPGESDCHLAEVWCGSERPVADNERMFDVLQRFGSQRNEVRFFL
RHERPPGRDIVSGPRSQDPSLKRNGVKVPGEYRRKENGVNSPRMDLTLAELQEMASRQQQQIEAQQQLLATKEQRLKFLK
QQDQRQQQQVAEQEKLKRLKEIAENQEAKLKKVRALKGHVEQKRLSNGKLVEEIEQMNNLFQQKQRELVLAVSKVEELTR
QLEMLKNGRIDSHHDNQSAVAELDRLYKELQLRNKLNQEQNAKLQQQRECLNKRNSEVAVMDKRVNELRDRLWKKKAALQ
QKENLPVSSDGNLPQQAASAPSRVAAVGPYIQSSTMPRMPSRPELLVKPALPDGSLVIQASEGPMKIQTLPNMRSGAASQ
TKGSKIHPVGPDWSPSNADLFPSQGSASVPQSTGNALDQVDDGEVPLREKEKKVRPFSMFDAVDQSNAPPSFGTLRKNQS
SEDILRDAQVANKNVAKVPPPVPTKPKQINLPYFGQTNQPPSDIKPDGSSQQLSTVVPSMGTKPKPAGQQPRVLLSPSIP
SVGQDQTLSPGSKQESPPAAAVRPFTPQPSKDTLLPPFRKPQTVAASSIYSMYTQQQAPGKNFQQAVQSALTKTHTRGPH
FSSVYGKPVIAAAQNQQQHPENIYSNSQGKPGSPEPETEPVSSVQENHENERIPRPLSPTKLLPFLSNPYRNQSDADLEA
LRKKLSNAPRPLKKRSSITEPEGPNGPNIQKLLYQRTTIAAMETISVPSYPSKSASVTASSESPVEIQNPYLHVEPEKEV
VSLVPESLSPEDVGNASTENSDMPAPSPGLDYEPEGVPDNSPNLQNNPEEPNPEAPHVLDVYLEEYPPYPPPPYPSGEPE
GPGEDSVSMRPPEITGQVSLPPGKRTNLRKTGSERIAHGMRVKFNPLALLLDSSLEGEFDLVQRIIYEVDDPSLPNDEGI
TALHNAVCAGHTEIVKFLVQFGVNVNAADSDGWTPLHCAASCNNVQVCKFLVESGAAVFAMTYSDMQTAADKCEEMEEGY
TQCSQFLYGVQEKMGIMNKGVIYALWDYEPQNDDELPMKEGDCMTIIHREDEDEIEWWWARLNDKEGYVPRNLLGLYPRI
KPRQRSLA

The percentage of ASPP2 null mice born is normal

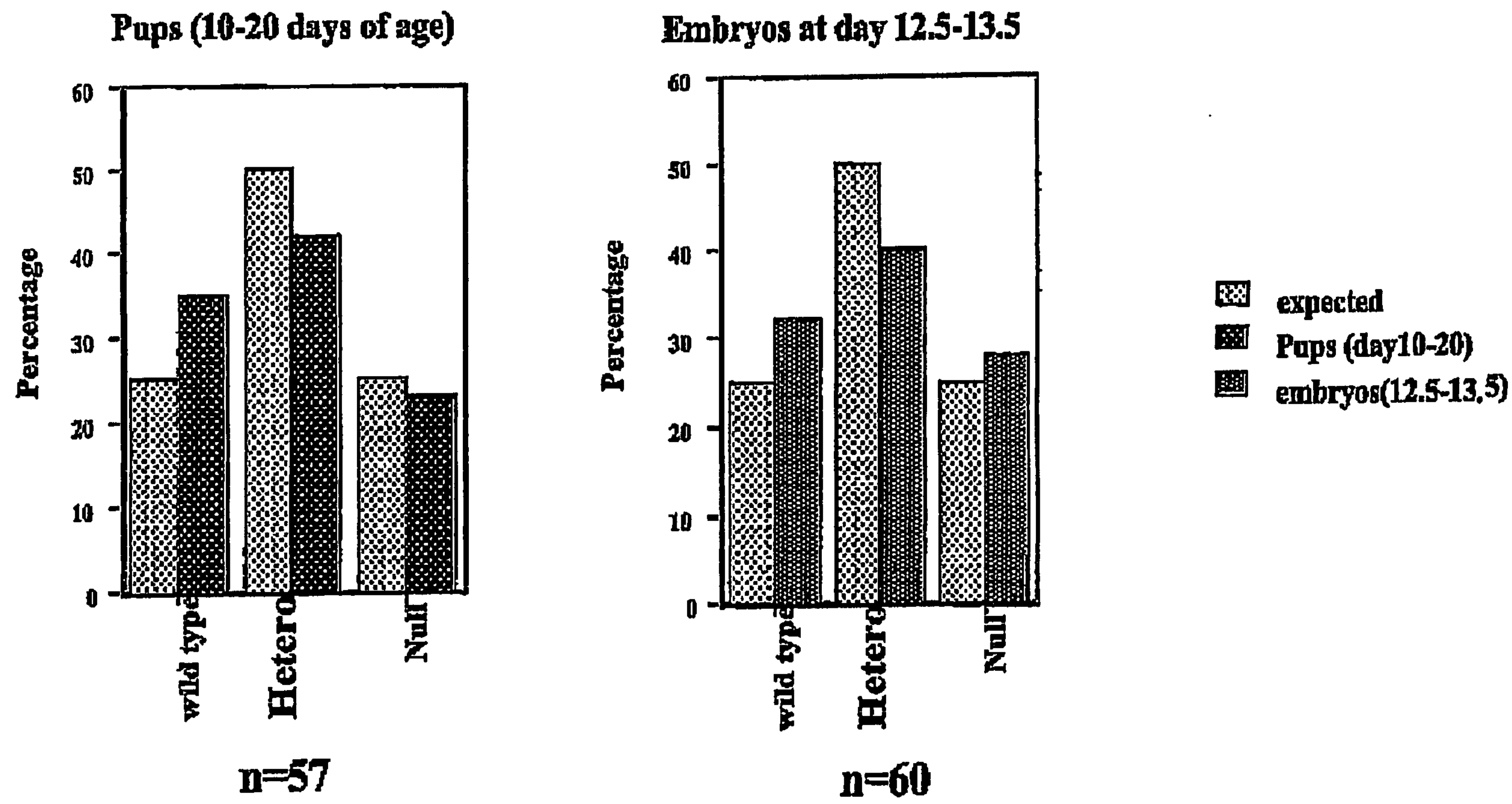


Figure 5

figure 6

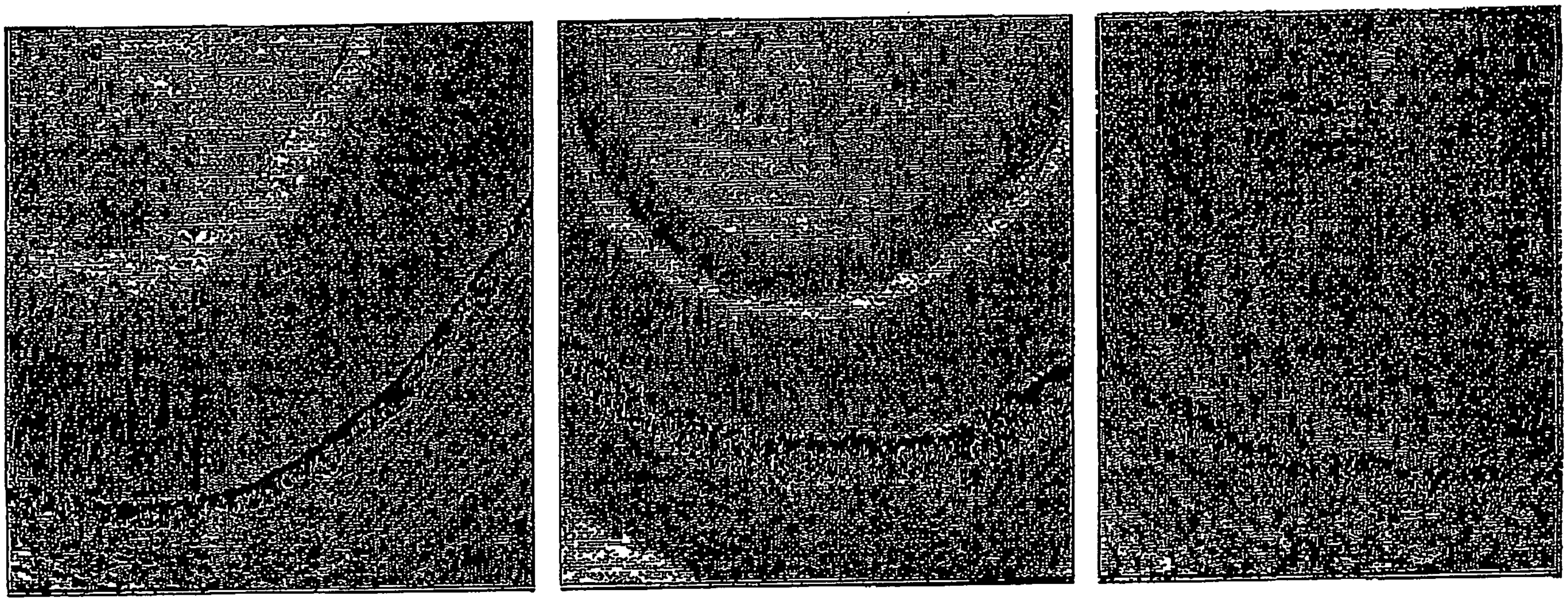
High Frequency of Malformation of ASPP2 Null Embryos

ASPP2 Null Mice Die Before Weaning

+/+ -/- -/- -/-

Figure 7

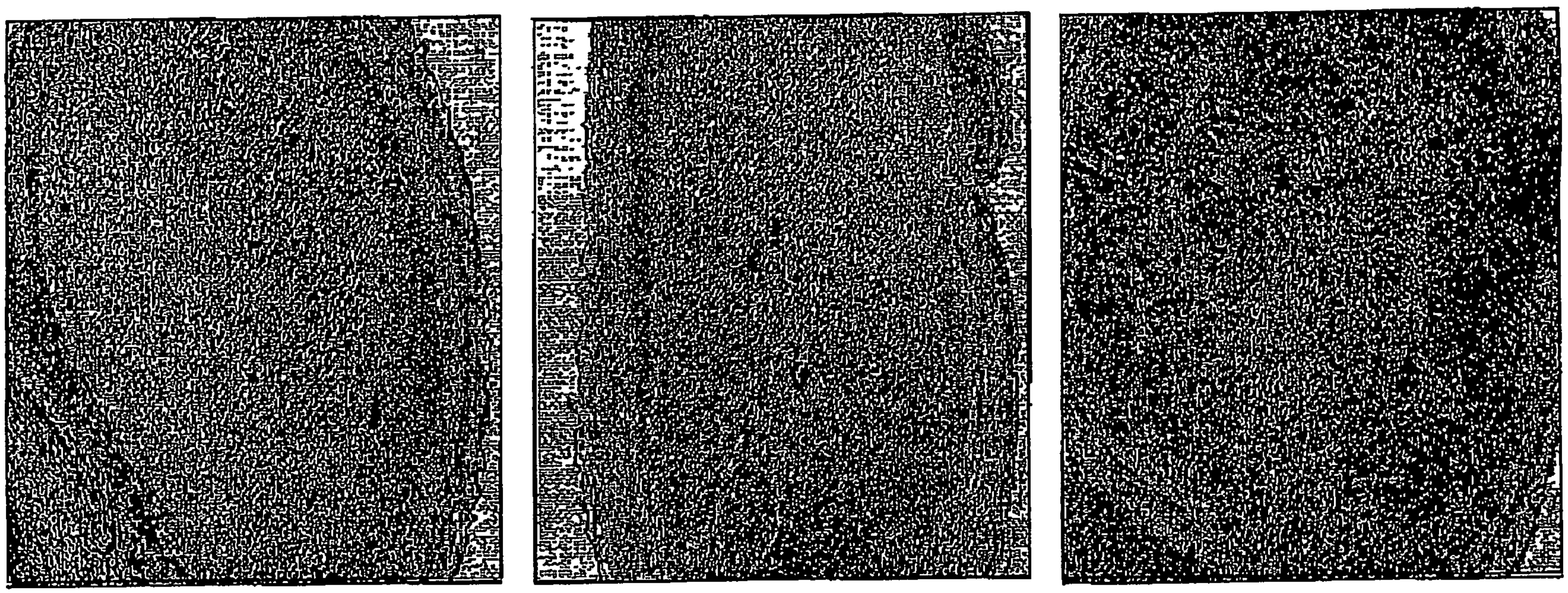
Eyes of day 13.5 ASPP2 embryos



+/+ (+/-) (-/-)

Figure 8

Brains of day 13.5 ASPP2 embryos



(+/+) (+/-) (-/-)

Figure 9

Abnormal growth in the brain of ASPP2 null embryo

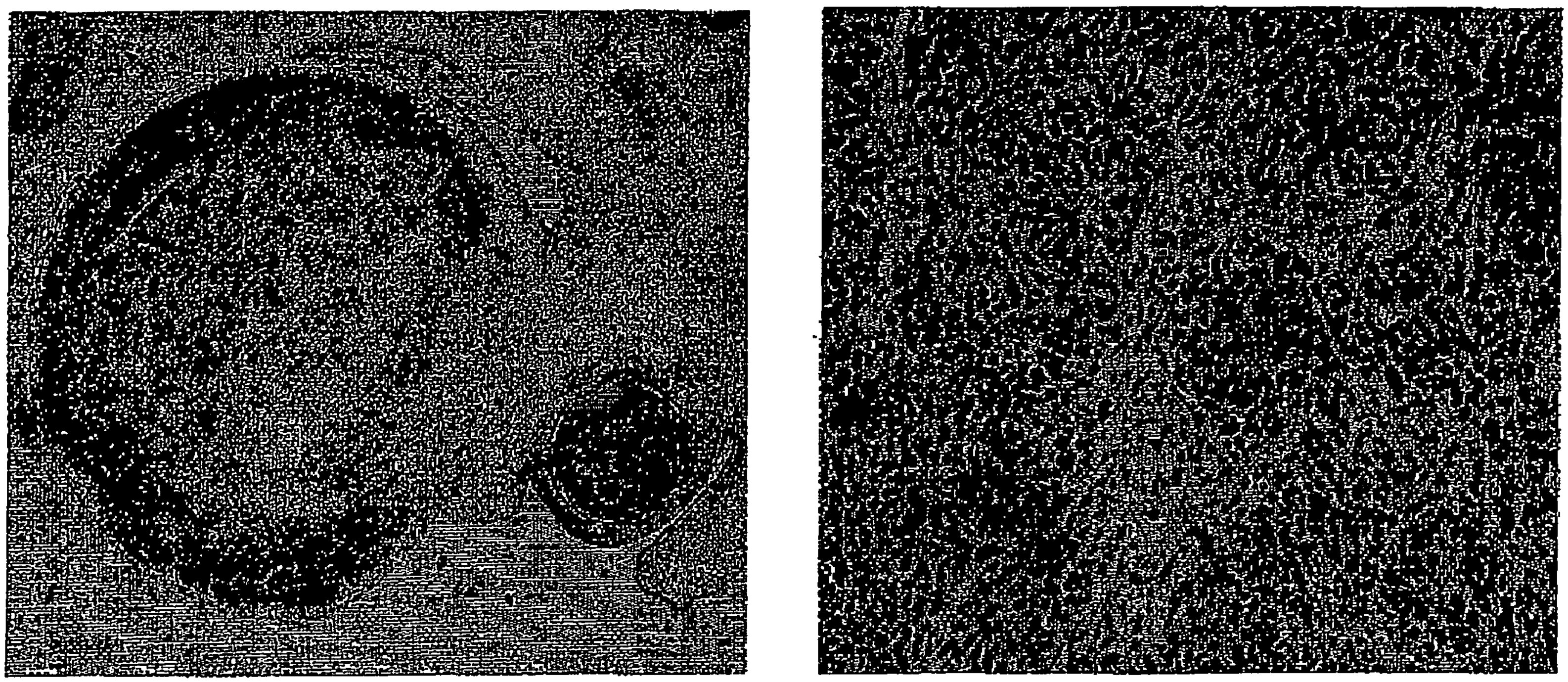


Figure 10